



RESEARCH ARTICLE

Effect of organic root-inducing substances on the propagation of katmon (*Dillenia philippinensis* Rolfe) in a mist clonal nursery chamber

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Abstract

The katmon (*Dillenia philippinensis* Rolfe) is a native species of the Philippines and is classified as “Vulnerable” on the IUCN Red List of Threatened Species due to the frequent cutting of trees for timber, leading to a significant decline in its population. The limited number of studies on the propagation of katmon underscores the need for further research to support its rapid reproduction, conservation and restoration. Clonal propagation allows the mass production of genetically homogeneous and high-quality plants. Natural exogenous rooting hormones (e.g., coconut water, honey and Aloe vera) promote root formation in cuttings by promoting cell division and differentiation, which leads to the development of new root primordia. The study evaluated various sources of katmon cuttings (Factor A), each treated with different organic root-inducing substances (Factor B), over a period of 90 days in a mist clonal chamber using 4 × 4 factorial experimental design. Results indicated that plagiotropic cuttings derived from cloned katmon plants and treated with Aloe vera gel outperformed most other treatments. These cuttings exhibited the highest survivability rate (90.0 %) after 30 days, which remained the highest at 60 (46.67 %) and 90 (30.0 %) days after planting. Furthermore, Aloe vera significantly influenced the number of leaf sprouts observed at 30 days (1.53). At 60 days, plagiotropic (1.67) and orthotropic (1.48) cuttings from cloned mother plants showed better performance than those derived from seed-germinated ones. Plagiotropic cloned cuttings remained impactful in terms of both the number (37.2) and length (33.2 cm) of roots at 90 days. The data generated from this study offer valuable insights for crafting effective propagation protocols for katmon. Such protocols can aid foresters, farmers and other stakeholders by increasing the population growth and supporting the conservation management of katmon.

Keywords: endemic tree species; growth performance; natural rooting enhancer; propagation technique; survivability

Introduction

The Philippines is home to approximately 3600 native tree species, 67 % of which are endemic, meaning they are found exclusively within the country's archipelago (1). One of these is the katmon (*Dillenia philippinensis* Rolfe), also known as the “Elephant apple”. This native plant thrives well in the low land to medium forest areas of the Philippines and grows up to 17 m high and 60 cm in diameter (2-4). Its edible fruit is commonly used to make jam and as a flavoring ingredient in dishes such as fish *sinigang*. It is also used for ornamental use, backyard planting, hedging, home gardening, urban greening, live fences and as a timber trade species (5).

However, this native tree was categorized as “Vulnerable” in 1998 by the IUCN Red List of Threatened Species and is currently assessed as “Near Threatened”. This decline is attributed to various human activities, such as firewood collection, poaching, quarrying, shifting cultivation, illegal logging and mining (2, 6-8). Given its ecological, cultural and economic importance, the conservation, monitoring,

reproduction and sustainable utilization of this species must be prioritized (6).

Due to the rapid decline in the population of the katmon species in lowland and forest areas, there is an urgent need to increase its propagation. Katmon species have a critical role in the ecosystem and other processes that keep the integrity of the forest (7). However, katmon is primarily propagated through seeds, which may not allow for rapid reproduction due to both internal and external limiting factors such as seed viability, temperature, and moisture conditions. These challenges underscore the need for alternative propagation methods, such as clonal propagation, which aim to reproduce the endangered species massively (8).

Several studies have successfully used cloning propagation to conserve other endangered species and promote their rapid reproduction, including *Ruta macrocarpa*, *Magnolia sirindhorniae*, *Chlorophytum arundinaceum* and *Paris polyphylla* var. *yunnanensis* (9-12). It has proven that cloned propagation has been highly effective as compared to seed

germinated such as in cloned *Eucalyptus* obtaining raw materials and paper mill achieved 135 % productivity and *Gmelina*, which showed improved the quality of timber by reducing the forking (13, 14).

Clonal propagation is a valuable mass propagation technique for producing high-quality, disease-free seedlings and conserving endangered plant species. Additionally, this method is useful to plant species that struggle to reproduce sexually, ensuring their genetic integrity and that they can be produced at any time of the year in a controlled environment. Clonal seedlings have a fundamental role in meeting the countrys' growing demand for timber and forest resources. Currently, Aklan State University (ASU), in partnership with the Department of Environment and Natural Resources (DENR), has established a hedge garden and mist clonal nursery project within the University to produce up to 60000 cloned seedlings for reforestation project programs. This initiative aims to consistently generate high-quality planting materials for various endemic, exotic, vulnerable and endangered species while integrating research into diverse propagation techniques to enhance the rapid reproduction of these species (15).

Despite its advantages, clonal propagation faces challenges such as low survivability and high mortality rates, often caused by external and internal factors. One of these factors is using incorrect concentrations of synthetic plant-regulating hormones such as auxin (Indole-3-butyric acid (IBA) and Naphthaleneacetic acid (NAA)) (16). These hormones play a crucial role by enhancing rapid and robust root development, vital for the survivability of the cloned plants (17, 18).

Although effective, synthetic hormones pose environmental risks, are costly and may have toxic effects (19). Consequently, several studies have investigated organic rooting agents as sustainable, cost-effective alternatives with lower ecological impact (20, 21). Moreover, more comprehensive and species-specific studies are needed, as plant responses to rooting agents can vary widely (19, 22, 23).

Natural rooting agents, such as coconut water, honey and Aloe vera, provide an eco-friendly and sustainable solution. These substances lower external input costs, improve root quality and enhance crop growth by supplying natural hormones (19). They provide a budget-friendly, safe and eco-friendly substitute for synthetic plant growth hormones like IBA, making them an excellent choice for horticultural crop propagation (24). Despite the economic and culinary significance, there was limited literature and no in-depth research was conducted on the asexual propagation of katmon, necessitating further research to increase its population (25).

This study aims to determine the effects of various organic root-inducing substances on the katmons' survivability and growth parameters. Understanding the physiological mechanisms of unrooted cuttings and the influence of both

endogenous and exogenous hormones is essential for the effective propagation of katmon (26). This knowledge supports nursery management in producing cloned katmon, facilitating the mass production of high-quality planting materials and contributing to the countrys' growing demand for wood and forest-based resources.

The study focuses on evaluating the impact of various organic root-inducing substances on the survivability and growth parameters of katmon cuttings, both cloned and seed-germinated, within a mist-clonal nursery chamber.

Materials and Methods

Research design

A complete randomized design (CRD) was employed in the study to propagate katmon using various sources of cuttings (Factor A), each treated with different organic root-inducing substances (Factor B), within a mist clonal chamber. The experiment followed a 4 × 4 factorial arrangement, replicated three times. Each treatment was planted with 20 katmon cuttings measuring 6–8 inches long and containing three nodes. The different sources of katmon cuttings and the corresponding root-inducing substances used in the study are shown in Table 1.

Collection of katmon cutting

All katmon cuttings were obtained from cloned and seed-germinated mother trees located at La Granja Farm, Brgy. Lapnag Banga, Aklan. This farm is one of the beneficiaries of cloned katmon cuttings distributed by the Aklan State University Clonal Nursery Project. Two types of cuttings were used in the study: plagiotropic and orthotropic. Orthotropic stem grows vertically (upright) and producing upward vegetative branches known as orthotropic shoots exhibiting monopodial development. Meanwhile, plagiotropic stems are from lateral branches growing horizontally showing sympodial growth.

All orthotropic and plagiotropic stems used in the study measuring approximately 6 to 8 inches in length and contained three nodes. The collected cuttings (both cloned and seed germinated) were labelled separately and placed in containers filled with water to maintain viability and prevent desiccation or water loss. The cuttings were then transported to the project site for further propagation and processing in the clonal mist nursery chamber.

Preparation of katmon cuttings

Excess leaves were trimmed off and 2 leaves were retained on each cutting, which were subsequently cut in half to minimize transpiration and reduce water loss. Excess leaves can lead to higher water loss and desiccation of roots. Hence, trimming the leaves benefits the cuttings, as it optimizes resource allocation and redirects its nutrients toward the roots instead of

Table 1. Sources of katmon cuttings with different root initiating substances

Factor A: Sources of katmon cuttings	Factor B: Root initiating substances
A ₁ – Orthotropic (Cloned)	B ₁ - Control
A ₂ – Plagiotropic (Cloned)	B ₂ - Aloe vera gel (<i>Aloe barbadensis</i> Miller) 100 mL with 1 L of water
A ₃ – Orthotropic (Seed germinated)	B ₃ - Coconut (<i>Cocos nucifera</i>) Water 100 mL with 1 L of water
A ₄ – Plagiotropic (Seed germinated)	B ₄ - Honey extracts 100 mL with 1 L of water

maintaining another biological process (27, 28). Moreover, this ensures that there is sufficient moisture for the root initiation.

The basal portion (cambium layer) of each cutting was scraped around 1-2 cm to stimulate root formation by repairing the damage. Scraping the stem damages the outer cortex, triggering the auxin accumulation, promoting cell differentiation and adventitious root formation (29, 30). This practice is widely used in other asexual propagation, such as air layering, grafting and budding (31).

All trimmed leaves and scraped cuttings were placed in the pail with the water, hydrating the cuttings to maintain cellular hydration and tissue variability, ensuring continuous water uptake, preventing air bubble (xylem embolism) and reduces the plant shock (32, 33).

Preparation of organic root initiating substances

Aloe vera was obtained from Tangalan, Aklan and was extracted using a mortar and pestle to obtain 100 mL of Aloe vera gel and mixed with 1 L of water. Previous studies have shown that fresh young coconut water, particularly from 7–9 months old, effectively promotes root induction and development. Hence, locally available fresh young coconut water from 7 months old coconut was diluted in 1 Liter of water (34). Meanwhile, raw and unpasteurized honey that was locally produced was utilized as a treatment in the study, measuring 100 mL with 1 L of water.

Katmon cutting was soaked in the prepared organic root-inducing solutions for 60 minutes before planting in the mist clonal nursery chamber. After soaking, the katmon cutting were planted in elevated propagation beds filled with the sterilized rooting media, maintaining a planting distance of 8 cm x 8 cm. Installed at the top of the chamber is the sprinkler water technology, which helps optimize the area to be watered. Irrigation was performed twice a day (morning and afternoon) until 90 days after planting.

Experimental environment condition

Weekly temperature and relative humidity were recorded using a digital humidity sensor and temperature to ensure proper growth and development of the cuttings and to prevent desiccation (35). Excessive humidity can promotes fungal growth, while high temperature may increase transpiration rate, potentially leading to wilting and plant mortality (36).

Data collection

Percent survivability

Percent survivability was computed by dividing the number of surviving cuttings by the total number of cuttings planted and multiplied by 100, as shown below:

$$\text{Percent survivability} = \frac{\text{Number of cuttings}}{\text{Total cuttings planted}} \times 100$$

(Eqn.1)

Growth performance

Growth parameters were gathered from 15 sample plants per treatment in each replicate. The total number of sprouts, as well as the number and length of leaves, were counted manually at 30, 60 and 90 days after planting. In terms of root

parameters, destructive sampling was conducted by carefully uprooting the katmon cuttings and manually counting the roots 90 days after planting. The root length was also measured at this stage by using a ruler (cm) from the base to the tip of the root.

Statistical analysis

The data collected were analyzed using a traditional two-way ANOVA to assess the interaction and main effects of the sources of katmon cuttings and root-inducing substances on the growth performance variables. A two-way ANOVA was performed, as this is suitable when examining the effect of two independent factors and their interactions. Then, pairwise comparisons were made using Scheffe test at a 5 % significance level of to identify statistically significant differences among treatments while conservatively controlling the family-wise error rate.

For independent variables with non-normally distributed residuals and heterogeneous variance-length of sprouts (90 days), number of leaves (90 days), length of leaves (90 days), number of roots (90 days), length of roots (90 days)-the Aligned Rank Transformation (ART) method was applied. ART is a non-parametric alternative suitable for factorial designs, preserving the interpretability of interaction effects under violations of ANOVA assumptions.

Following the ART transformation, a two-way ANOVA was performed to further explore the effects of cutting sources and root-inducing substances on these variables.

Subsequent pairwise comparisons were conducted using estimated marginal means, with p-values adjusted for multiple comparisons using Tukey's Honest Significant Difference (HSD) method to control family-wise error rate.

All statistical analyses were conducted using R software (version 4.2.3, R Core Team, 2023), ensuring accuracy and reproducibility of results. Survivability percentage data, being straightforward, was analyzed using Microsoft Excel as it did not require advanced statistical modelling.

Results and Discussion

Survivability

Fig. 1 shows the katmons' percent survivability from different cutting sources subjected to different root-inducing substances at 30 DAP. Katmon cuttings from plagiotropic cloned treated with Aloe vera displayed the highest survivability (90.0 %). Following closely were plagiotropic cloned cuttings treated with honey extract (88.89 %), while the control group recorded a survivability of 78.89 %. Orthotropic cloned cuttings demonstrated favourable survivability, especially those soaked in coconut water (80.0 %) and the least in honey extract (70.0 %).

In contrast, plagiotropic cuttings from seed-germinated sources displayed significantly lower survivability, with those treated with coconut water recording the highest rate within this group (30.0 %). Orthotropic cuttings from seed-germinated sources exhibited the lowest survivability overall, with the highest recorded at only 10 % in the control group. Overall, plagiotropic cloned cuttings outperformed all other cutting types in terms of survivability and cloned cuttings

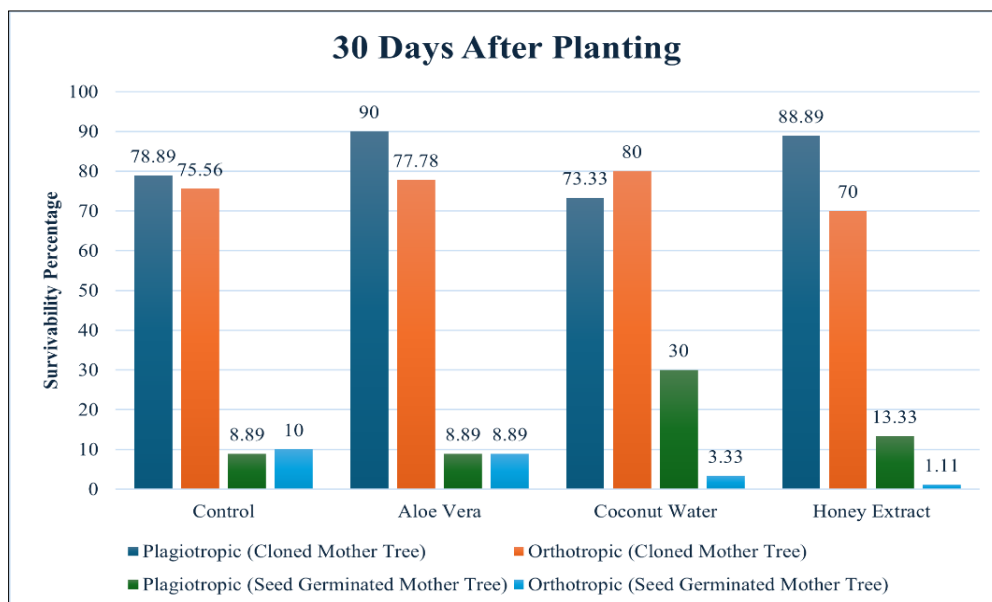


Fig. 1. Survivability percentage of katmon (*Dillenia philippinensis* Rolfe) cuttings 30 days after planting

demonstrated greater survivability than seed-germinated cuttings after 30 days. After 60 days (Fig. 2), there was a significant decline in survivability across all treatment groups. Some group showing complete lack of survival, particularly the plagiotropic seed-germinated in the control group and the orthotropic seed-germinated cuttings treated with Aloe vera and honey extract. Despite this overall decline, plagiotropic cloned cuttings treated with Aloe vera recorded the highest survivability (46.67 %).

By 90-day mark (Fig. 3), there was a continuous decline in survivability across all treatment groups, except for orthotropic cloned cuttings in the control group, which maintained a survivability of 12.22 %. Plagiotropic cloned cuttings treated with Aloe vera remained consistent with the highest survivability (30.0 %). It is worth noting that after 90 days, all orthotropic seed-germinated cuttings exhibited complete zero survivability. This observation is aligned with the conclusion that plagiotropic cuttings of semi-hardwood trees containing four (4) leaves treated with rooting hormones could perform better than the four-leaf cuttings of orthotropic of the same species (37).

The data on the experimental environmental condition recorded showed that the average temperature of the mist clonal nursery chamber ranged from 26 to 28 °C, with average relative humidity levels between 87 % and 90 %. Temperatures have a significant influence on root formation. Previous studies have shown that the optimal rooting temperatures for most plant species' ranges from 21 °C to 26 °C (38). However, the data shows that the experiments' environmental condition exceeded this ideal range, which may cause a high mortality rate observed. High temperatures can increase the transpiration rate and water loss, which may lead to necrosis and death of the plant (39).

On the other hand, relative humidity levels of 85 %–90 % are widely recommended in clonal chambers to reduce the risk of fungal growth and dehydration. The recorded humidity levels were within the recommended range, which likely helped mitigate some environmental stress despite the elevated temperatures (40, 41).

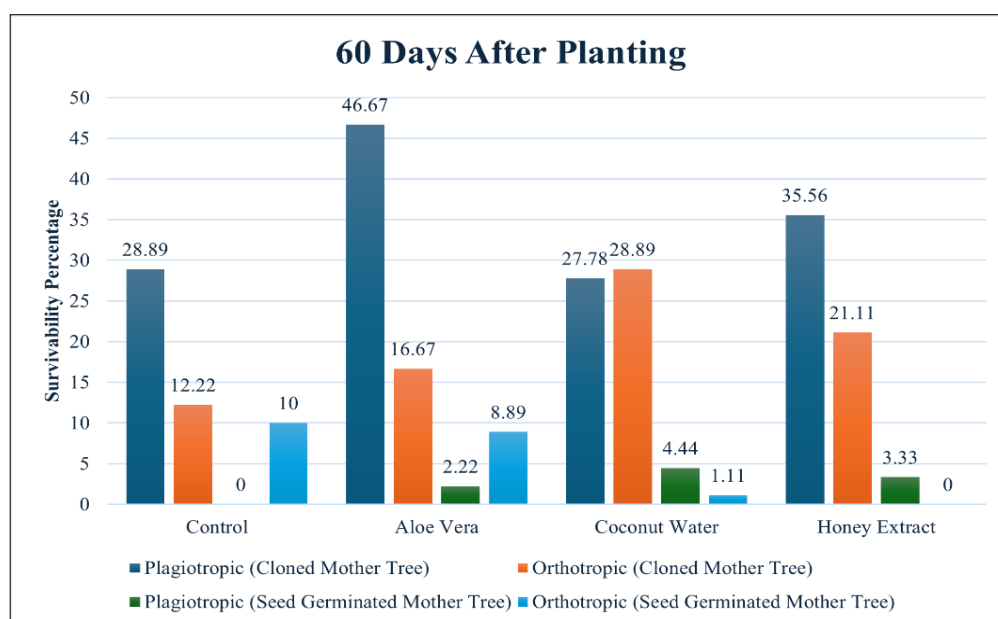


Fig. 2. Survivability percentage of katmon (*Dillenia philippinensis* Rolfe) cuttings 60 days after planting.

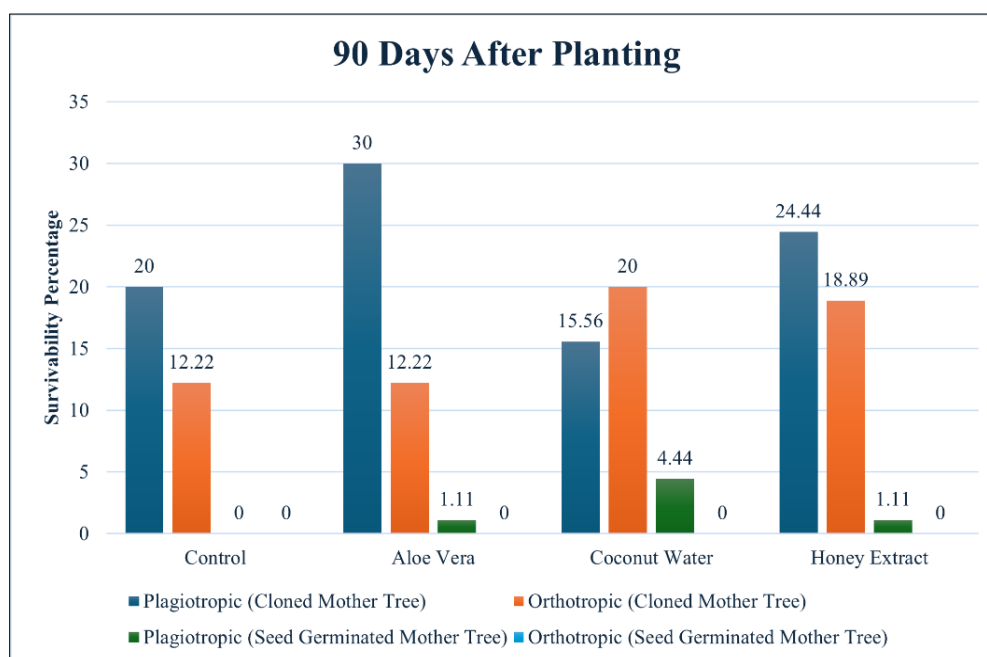


Fig. 3. Survivability percentage of katmon (*Dillenia philippinensis* Rolfe) cuttings 90 days after planting.

Number of leaf sprouts

Table 2 presents the results of a two-way analysis of variance (ANOVA) conducted to assess the number of leaf sprouts in katmon cuttings from various sources subjected to different organic root-inducing substances at 30 and 60 days after planting. At 30 DAP, the findings reveal no significant difference in the number of leaf sprouts among the different sources of katmon cuttings ($p = 0.2513$). However, a significant difference was observed among the root-inducing substances ($p = 0.0137$). Specifically, cuttings treated with Aloe vera gel produced a significantly higher number of leaf sprouts, with a mean of 1.53, compared to cuttings treated with honey, which had a mean of 1.04 leaf sprouts, as shown in Table 3. Previous studies have demonstrated that Aloe vera extracts effectively induce multiple shoots in pomegranate, dragon fruit and other plant cuttings (42, 43). This is attributed to the presence of natural plant hormones in Aloe vera, including auxins (e.g., Indole-3-Acetic Acid) and gibberellins, which promote cell differentiation, division and elongation, thereby enhancing shoot and growth development (44).

The analysis further revealed no significant interaction effect between the source of katmon cuttings and root-inducing substances on the number of leaf sprouts ($p = 0.1462$) (Table 2). Post hoc analysis confirmed that Aloe vera gel significantly increased the number of leaf sprouts compared to all other treatments, indicating a strong positive influence on shoot development. Conversely, honey extracts resulted in the lowest number of sprouts and were significantly different from the control group. Honey extracts alone do not induce shoot formation in plant cuttings (45). Honey primarily serves as an

antimicrobial agent, as it contains enzymes such as glucose oxidase that produce hydrogen peroxide (H_2O_2), along with organic acids like gluconic acid and polyphenols (flavonoids), which inhibit microbial growth (46-49). Therefore, while honey protects cuttings from pathogens, it does not directly influence shoot formation.

Aloe vera and indole-3-butyric acid (IBA) have been shown to positively affect sprouting in plant cuttings by promoting root initiation, reducing fungal rot and increasing the overall success rate of propagation (50, 51). These substances also enhance development and alter the bioactive composition of various plant species by supplying a unique combination of nutrients essential for growth (52).

At 60 DAP, a significant difference in the number of leaf sprouts was observed among the different sources of katmon cuttings ($F(3, 47) = 21.068$, $p < .001$) (Table 2). Plagiotropic cloned cuttings exhibited the highest mean number of leaf sprouts ($M = 1.67$), closely followed by orthotropic cloned cuttings ($M = 1.48$). Both groups of cloned cuttings significantly outperformed seed-germinated cuttings. Notably, orthotropic seed-germinated cuttings exhibited no leaf sprouting, regardless of the root-inducing substance applied. This finding supports the assertion that clonal cuttings enable more rapid and uniform shoot development and are more effective in propagating woody plant species (53). The data suggest that plagiotropic and orthotropic cloned cuttings demonstrate strong juvenile vigor, facilitating cell division and differentiation that promote shoot development in katmon (54, 55).

Table 2. Two-way analysis of the variance of number of sprouts of katmon (*Dillenia philippinensis* Rolfe) among different sources of katmon cuttings subjected to different levels of root initiating substances 30 days and 60 days after planting

Sources of variation	df	30 DAP		60 DAP	
		F	p-value	F	p-value
SKC	3	1.43	0.2513	21.068	<0.001*
RIS	3	4.14	0.0137*	0.868	0.468
SKC × RIS	9	1.64	0.1462	0.194	0.993
Error	32				
Total	47				

SKC - Sources of Katmon Cuttings; RIS- Root Initiating Substances *Significant at 5% level

Table 3. Pairwise means comparison of sources of katmon (*Dillenia philippinensis* Rolfe) cuttings on number of sprouts 30 and 60 days after planting

Sources of katmon cuttings	30 DAP				60 DAP				Mean*
	B1	B2	B3	B4	B1	B2	B3	B4	
A1	1.56	1.47	1.47	1.25	1.43	1.42	2.02	1.82	1.67 ^a
A2	1.20	1.29	1.05	1.22	1.25	1.58	1.63	1.48	1.48 ^a
A3	0.69	1.49	1.33	1.04	0.58	0.67	1.04	0.87	0.79 ^b
A4	0.89	1.89	1.33	0.67	0.00	0.00	0.00	0.00	0.00 ^c
Mean*	1.08 ^{ab}	1.53 ^a	1.29 ^{ab}	1.04 ^b					

A1-Plagiotropic Cloned; B1-Control; A2-Orthotropic Cloned; B2-Aloevera Gel; A3-Plagiotropic Seed Germinated; B3-Coconut; A4-Orthotropic Seed Germinated; B4-Honey Extracts

Means that do not share the same letter are significantly different at 5% level of significant using Scheffe's Test. Means that do not share the same letter are significantly different at 5% level of significant using Scheffe's Test.

In contrast, seed-germinated cuttings failed to produce any leaf sprouts. Some studies indicate that seed-derived cuttings typically exhibit lower endogenous auxin levels, which limits their ability to initiate new shoot growth (56, 57). Cloned cuttings, being genetically identical to the parent plant, bypass the seedling or juvenile phase, thereby accelerating both shoot and root development (58). Research further supports that cloned cuttings demonstrate vigorous vegetative traits, such as rapid leaf sprouting (59) and many species propagated through cloning have shown enhanced shoot production and better adaptability to environmental stresses (19, 60).

The enhanced shoot development observed in cloned katmon cuttings may also be attributed to ecological advantages conferred by clonal propagation. These advantages include improved access to water, nutrients and light in fragmented habitats, physiological integration between shoots, spatial adaptability, reduced mortality risk and rapid colonization of new areas. While propagation from seeds can promote genetic diversity, it is often hindered by various biochemical, physical and morphological barriers (61, 62).

Moreover, statistical analysis indicated no significant differences in the number of leaf sprouts among the various root-inducing substances applied at 60 DAP ($p = 0.468$) and no significant interaction effect was found between the cutting sources and the root-inducing substances ($p = 0.993$) (Table 2). Root development in tree species is often influenced more by internal factors such as endogenous auxin levels, carbohydrate reserves and environmental stimuli, rather than by external rooting treatments (54, 63).

Pairwise mean comparisons (Table 3) further confirmed that katmon cuttings sourced from cloned mother trees-both plagiotropic and orthotropic-produced a significantly greater number of leaf sprouts than those from seed-germinated sources. This is likely due to factors such as genetic uniformity, physiological maturity and the juvenile state of the cuttings (53, 57).

Length of leaf sprouts

The length of the leaf sprouts 30 days after planting was examined and the results indicated that no significant effect was observed among different sources of katmon cuttings (p -value = $0.939 > 0.05$) (Table 4). The mean length of the leaf sprouts ranged from 1.42 to 2.18 and all values were comparable. Similarly, no significant differences were observed in the length of leaf sprouts produced by katmon when subjected to different levels of root-inducing substances (p -value = $0.262 > 0.05$). The recorded length of the sprouts varied between 1.42 and 2.18, as shown in Table 5. Additionally, no interaction effect was observed between different cuttings sources and various root-inducing substance levels (p -value = $0.720 > 0.05$). These results suggest that the sources of katmon cuttings and the applied root-inducing substances and their interaction did not significantly impact the length of the sprouts within the 30-day study period. It is plausible that the growth patterns of katmon cuttings exhibited uniformity during this timeframe.

The study further indicates that katmon leaf length shows no measurable influences and that sprouts have uniform lengths among sources of cuttings. This may be due to its inherent physiological traits showing consistent shoot growth in the early stage of sprouting and other endogenous reserves in the plant (64). Endogenous reserves store energy in the cuttings, which support their regeneration and plant growth, katmon cuttings may have enough carbohydrates stored and endogenous reserves allow them to produce a consistent length of sprouts (65). Studies have shown stored carbohydrates have a critical role in root and shoot development (66). Early sprout elongations of tree species are highly dependent on internal factors such as endogenous reserves and carbohydrates rather than external stimulants (67).

Thus, the lack of significant effects from root-inducing substances may be due to their limited role in shoot elongation during early development (68). Several studies have conducted asexually propagated tree species that used root-inducing

Table 4. Two-way analysis of variance of length of sprouts of katmon (*Dillenia philippinensis* Rolfe) among different sources of katmon cuttings subjected to different levels of root initiating substances 30, 60 and 90 days after planting

Variation	df	30 DAP		60 DAP		90 DAP	
		F	p-value	F	p-value	F	p-value
SKC	3	0.133	0.939	15.67	<0.001*	14.947	<0.001*
RIS	3	1.395	0.262	0.74	0.535	4.323	0.011*
SKC × RIS	9	0.681	0.720	0.20	0.993	2.509	0.027*
Error	32						
Total	47						

SKC – Sources of Katmon Cuttings; RIS- Root Initiating Substances *Significant at 5% level

Table 5. Sprout mean length (cm) of the different sources of katmon (*Dillenia philippinensis* Rolfe) cuttings influenced with different levels of root initiating substances 30 and 60 days after planting

Sources of katmon cuttings	Root initiating substances (30 DAP)				Mean ^{ns}	Root initiating substances (60 DAP)				Mean [*]
	B1	B2	B3	B4		B1	B2	B3	B4	
A1	1.59	1.46	1.03	1.58	1.42	1.96	2.42	2.43	2.07	2.22^a
A2	1.55	1.55	1.44	1.61	1.54	2.39	2.80	2.32	2.06	2.39^a
A3	1.98	1.70	2.53	1.14	1.84	0.98	1.62	1.77	1.44	1.45^a
A4	1.61	1.82	2.47	2.83	2.18	0.00	0.00	0.59	0.00	0.15^b

A1-Plagiotropic Cloned; B1-Control; A2-Orthotropic Cloned; B2-Aloe vera Gel; A3-Plagiotropic Seed Germinated; B3-Coconut; A4-Orthotropic Seed Germinated; B4-Honey Extracts

30 DAP: ns – not significant.

60 DAP: Means that do not share the same letter are significantly different at 5% level of significant using Scheffe's Test.

Sources of katmon cuttings	Root initiating substances				Mean ^{ns}
	B1	B2	B3	B4	
A1	1.59	1.46	1.03	1.58	1.42
A2	1.55	1.55	1.44	1.61	1.54
A3	1.98	1.70	2.53	1.14	1.84
A4	1.61	1.82	2.47	2.83	2.18

Pairwise comparison of sources of katmon (*Dillenia philippinensis* Rolfe) cuttings on length of sprout 60 days after planting.

Sources of katmon cuttings	Root initiating substances				Mean [*]
	B1	B2	B3	B4	
A1	1.96	2.42	2.43	2.07	2.22^a
A2	2.39	2.80	2.32	2.06	2.39^a
A3	0.98	1.62	1.77	1.44	1.45^a
A4	0.00	0.00	0.59	0.00	0.15^b

A1-Plagiotropic Cloned; B1-Control; A2-Orthotropic Cloned; B2-Aloe vera Gel; A3-Plagiotropic Seed Germinated; B3-Coconut; A4-Orthotropic Seed Germinated; B4-Honey Extracts

ns – not significant; Means that do not share the same letter are significantly different at 5% level of significant using Scheffe's Test.

substances to increase root development rather than shoot development (19, 69). Moreover, shoot elongation often requires a longer observation period, as early stages may not reflect the full impact of external treatments (70, 71).

The findings indicated a highly influence p-value of less than 0.001 ($p < 0.001$) for the length of the leaf sprouts produced by different sources of cuttings 60 days after planting (Table 4). This suggests a positive effect on the sprouts' overall length among various cuttings sources. Pairwise comparison means revealed comparable mean length results for both cloned sources (plagiotropic and orthotropic) and seed-germinated plagiotropic cuttings, with lengths of 2.22 cm, 2.39 cm and 1.45 cm respectively. In contrast, seed-germinated orthotropic cuttings resulted in significantly shorter leaf sprouts, measuring only 0.15 cm (Table 5). Furthermore, no significant results were observed for the impact of root-inducing substances on the length of the sprouts during the 60-day period.

Similarly, statistical analysis showed no significant results regarding the interaction between different sources of cuttings and root-inducing substances in relation to leaf sprout length within the 60-day study period. Sprouting plays a crucial role in plant growth and development as it contributes to increased nutrient availability, improved digestion and reduced anti-nutrients.

The study indicates that longer leaf sprouts were observed among katmon cuttings at 60 days, indicating superior growth on cloned katmon (plagiotropic and orthotropic), outperforming the seed germinated cuttings in shoot elongation. Cloned cuttings have vigorous physiological traits identical to its parent plant having active meristem and endogenous content reserve factors in contributing to shoot and root development (72, 73). This highlights the importance of vegetative propagation from cloned cuttings, which does not

require external root initiating substances to develop and produce longer leaf shoots, reducing the cost of propagation. Cloned cuttings maintained ideal physiological genetic characteristics and avoided genetic variation from seed-germinated cuttings (74).

At 90 days after planting, both the interaction effect and main effects for sources of katmon cuttings and root-inducing substances were deemed significant, as evidenced by $F(9,47) = 2.509$, $p = 0.027$, $F(3,47) = 14.947$, $p = 0.011$, $F(3,47) = 4.323$, $p < 0.001$ respectively (Table 4). However, the pairwise comparison test revealed that only the main effects of sources of katmon cuttings and root-inducing substances were significant, with no indication of a significant interaction. While each factor had a notable impact on the length of leaf sprouts after 90 days. There were no specific combinations of sources and root-inducing substances which were significantly different. It is essential to acknowledge that the non-significant results in the pairwise comparison of the interaction effect may be influenced by the adjustments (Tukey's HSD) made to account for multiple comparisons.

Further exploration into the main effects of sources of katmon cuttings disclosed that orthotropic seed-germinated (EM = 7.5, SE = 3.13) significantly yielded shorter leaf sprout length compared to other sources of katmon cuttings. In contrast, plagiotropic cloned (EM = 35.2, SE = 3.13), orthotropic cloned (EM = 30.4, SE = 3.13) and plagiotropic seed-germinated (EM = 24.9, SE = 3.13) had similar effects on the length of leaf sprouts after 90 days. On the other hand, investigating the main effect of root-inducing substances revealed that katmon cuttings soaked with Aloe vera produced the longest sprouts (EM = 33.6, SE = 4.06), significantly higher than katmon cuttings from the control group (EM = 13.2, SE = 4.06) as shown in Table 6.

Table 6. Pairwise comparison of sources of katmon (*Dillenia philippinensis* Rolfe) cuttings and root-inducing substances on length of sprout 90 days after planting

Sources of katmon cuttings	Estimated mean (EM)	SE	df	Lower confidence interval	Upper confidence interval	
A1	35.2	3.13	32	28.80	41.5	a
A2	30.4	3.13	32	24.05	36.8	a
A3	24.9	3.13	32	18.55	31.3	a
A4	7.5	3.13	32	1.13	13.9	b

A1-Plagiotropic Cloned; A2-Orthotropic Cloned; A3-Plagiotropic Seed Germinated; A4-Orthotropic Seed Germinated; Estimated mean that do not share the same letter are significantly different at 5% level. Confidence level was set at 95% level. P-value adjustment was employed using Tukey's HSD method for comparing a family of 4 estimates.

Root initiating substances	Estimated mean (EM)	SE	df	Lower confidence interval	Upper confidence interval	
B1	13.2	4.06	32	4.9	21.4	b
B2	33.6	4.06	32	25.3	41.8	a
B3	25.7	4.06	32	17.4	33.9	ab
B4	25.6	4.06	32	17.3	33.8	ab

B1-Control; B2-Aloe Vera; B3-Coconut; B4-Honey Extract; Estimated mean that do not share the same letter are significantly different at 5% level. Confidence level was set at 95% level. P-value adjustment was employed using Tukey's HSD method for comparing a family of 4 estimates

Source of katmon cuttings x root initiating substances	Estimated mean (EM)	SE	df	Lower confidence interval	Upper confidence interval	
A1 B1	38.0	7.46	32	22.81	53.2	a
A1 B2	20.3	7.46	32	5.14	35.5	a
A1 B3	19.3	7.46	32	4.14	34.5	a
A1 B4	22.3	7.46	32	7.14	37.5	a
A2 B1	6.0	7.46	32	-9.19	21.2	a
A2 B2	28.3	7.46	32	13.14	43.5	a
A2 B3	25.7	7.46	32	10.48	40.9	a
A2 B4	41.3	7.46	32	26.14	56.5	a
A3 B1	22.3	7.46	32	7.14	37.5	a
A3 B2	30.0	7.46	32	14.81	45.2	a
A3 B3	32.0	7.46	32	16.81	47.2	a
A3 B4	16.3	7.46	32	1.14	31.5	a
A4 B1	41.3	7.46	32	26.14	56.5	a
A4 B2	14.0	7.46	32	-1.19	29.2	a
A4 B3	22.0	7.46	32	6.81	37.2	a
A4 B4	19.0	7.46	32	3.81	34.2	a

Estimated mean that do not share the same letter are significantly different at 5% level. Confidence level was set at 95% level. P-value adjustment was employed using Tukey's HSD method for comparing a family of 16 estimates.

Several studies have confirmed that cloned cuttings produced significant growth performance compared to seed-germinated plants (75, 76). Hence, cloned katmon cuttings significantly produced longer sprouts and highlighted its superior growth, continuously producing longer leaves than seed-germinated cuttings. Additionally, root-inducing substances such as Aloe vera have produced the longest leaf sprouts as compared to controlled cuttings influencing leaf elongation. Several studies have confirmed that Aloe vera has a significant effect and influences shoot development (19, 43).

Number of leaves

The results indicate that the interaction between sources of katmon cuttings and root-inducing substances did not have a significant impact on the number of leaves of katmon after 60 days, as demonstrated by $F(3,47) = 17.24$, $p < .001$ (Table 7). However, the main effect of sources of katmon cuttings did show an influence on the number of leaves. Plagiotropic cloned ($M = 1.56$) and orthotropic cloned ($M = 1.53$) exhibited a significantly higher number of leaves compared to plagiotropic seed-germinated ($M = 0.78$) and orthotropic seed-germinated ($M = 0.08$). Among seed-germinated katmon cuttings, plagiotropic ($M = 0.78$) showed a significantly higher leaf count than orthotropic ($M = 0.08$), as shown in Table 8. The data suggests that utilizing both cloned sources (both plagiotrophic and orthotropic) of katmon cuttings positively impacts

achieving a higher number of leaves. In contrast, various root-inducing substances did not significantly affect the number of leaves.

The study highlights that katmon propagated through cloned cuttings influences leaf production, which is vital for the growth and development of the plant. Studies documented that cloned cutting significantly increased the number of leaves such as *Eucalyptus camaldulensis*, *Hopea odorata* and *Cannabis sativa* (77-81). In contrast, organic root-inducing substances did not influence leaf production in different sources of cuttings due to its plants' physiological factors, such as matured meristematic tissues, rather than external growth stimulants (19, 82). Hence, the study underscores that propagating katmon through cloned cuttings is more efficient in producing more leaves, vital for biological and physiological processes for plant growth.

At 90 days after planting, no significant interaction was observed between the source of katmon cuttings and root-inducing substances on the leaf count after 90 days ($F(3,47) = 1.1702$, $p = 0.346$, (Table 7). However, the main effect of sources of katmon cuttings did show an influence on the number of katmon leaves ($F(3,47) = 32.238$, $p < .0001$). The estimated mean differences demonstrated significance, with plagiotropic cloned ($EM = 36.6$, $SE = 2.39$) differing significantly from both plagiotropic seed-germinated ($EM = 16.5$, $SE = 2.39$)

Table 7. Two-way analysis of the variance of number of leaves katmon (*Dillenia philippinensis* Rolfe) among different sources of katmon cuttings subjected to different levels of root initiating substances after 60 and 90 days after planting

Sources of variation	df	60 DAP		90 DAP	
		F	p-value	F	p-value
SKC	3	17.24	<0.001*	32.238	<0.001*
RIS	3	1.19	0.328	2.338	0.092
SKC × RIS	9	0.15	0.997	1.1702	0.346
Error	32				
Total	47				

SKC – Sources of Katmon Cuttings; RIS- Root Initiating Substances *Significant at 5% level

Table 8. Pairwise comparison of sources of katmon (*Dillenia philippinensis* Rolfe) cuttings on the number of leaves at 60 and 90 days after planting

Sources of katmon cuttings	60 DAP Mean	90 DAP Estimated mean	SE	df	Lower CI	Upper CI
A1	1.56 ^a	36.6 ^a	2.39	32	31.71	41.5
A2	1.53 ^a	35.4 ^a	2.39	32	30.54	40.3
A3	0.78 ^b	16.5 ^b	2.39	32	11.62	21.4
A4	0.08 ^c	9.5 ^b	2.39	32	4.62	14

A1-Plagiotropic Cloned; A2-Orthotropic Cloned; A3-Plagiotropic Seed Germinated; A4-Orthotropic Seed Germinated; Means that do not share the same letter are significantly different at 5% level of significant using Scheffe's Test (60 DAP). Estimated mean that do not share the same letter are significantly different at 5% level (90 DAP). Confidence level was set at 95% level. P-value adjustment was employed using Tukey's HSD method for comparing a family of 4 estimates.

and orthotropic seed-germinated (EM = 9.5, SE = 2.39). Similarly, orthotropic clones exhibited significant estimated mean differences with plagiotropic seed-germinated (EM = 16.5, SE = 2.39) and orthotropic seed-germinated (EM = 9.5, SE = 2.39), as shown in Table 8.

The study highlights the superiority of both plagiotropic and orthotropic cloned cuttings, producing a higher number of leaves than seed-germinated cuttings. Seed germinated cuttings exhibited delayed growth, particularly in leaf count, primarily due to slow physiological maturity and energy reserves in the plant. Seed germinated cuttings are independent of their endogenous reserves, while cloned cuttings inherent their nutrients, as well as genetic and physiological traits from the parent plant (55). Cloned cuttings tend to have the same physiological maturity as their identical parent, which enhances the plants' vegetative growth and overall vigour (83).

Meanwhile, the study supports the conclusion that organic root-inducing substances did not have significant influence on leaf production, as their primary function is root development rather than shoot development. For instance, several studies have used organic initiating substances to promote roots and do not address shoot or leaf development (19, 43).

Length of leaves

The length of leaves after 60 days was not significantly influenced by the interaction between the source of katmon cuttings and root-inducing substances, as indicated by F (3, 47)

= 0.14, p = 0.998 (Table 9). However, the main effect of the sources of katmon cuttings significantly impacted the length of katmon leaves F (3, 47) = 9.57, p < .0001. Specifically, orthotropic seed germination resulted in significantly shorter sprouts (M = 0.29) compared to other sources of katmon cuttings. Among the remaining sources, orthotropic cloned exhibited the highest effect (M = 3.06), followed by plagiotropic cloned (M = 2.72) and then plagiotropic seed-germinated (M = 2.14), as shown in Table 10.

The length of leaves after 90 days did not show a significant interaction between the sources of katmon cuttings and root-inducing substances, as evidenced by F (3, 47) = 0.718, p = 0.689 (Table 9). However, the main effect of sources of katmon cuttings did influence the length of katmon leaves (F (3, 47) = 12.639, p < .0001). The estimated meaning differences revealed significance, with orthotropic cloned (EM = 34.5, SE = 3.26) differing significantly from both plagiotropic seed-germinated (EM = 21.2, SE = 3.26) and orthotropic seed-germinated (EM = 9.5, SE = 3.26). Additionally, the estimated mean differences showed significance, with plagiotropic cloned (EM = 32.8, SE = 3.26) differing significantly from orthotropic seed-germinated (EM = 9.5, SE = 3.26), as shown in Table 10. On comparing cloned cuttings, both plagiotropic and orthotropic yielded a similar response. Similarly, on comparing seed-germinated cuttings, both plagiotropic and orthotropic exhibited the same response.

The study emphasized that orthotropic and plagiotropic cloned cuttings were consistent in exhibiting leaf development in katmon, particularly in leaf production and

Table 9. Two-way analysis of variance of length of sprouts of katmon (*Dillenia philippinensis* Rolfe) among different sources of katmon cuttings subjected to different levels of root initiating substances 60 and 90 days after planting

Sources of variation	df	60 DAP		90 DAP	
		F	p-value	F	p-value
SKC	3	9.57	<0.0001*	12.639	<0.0001*
RIS	3	0.85	0.478	1.529	0.2258
SKC × RIS	9	0.14	0.998	0.718	0.6887
Error	32				
Total	47				

SKC – Sources of Katmon Cuttings; RIS- Root Initiating Substances *Significant at 5% level. The 90 DAP were analyzed using the Aligned Rank Test

Table 10. Pairwise comparison of sources of katmon (*Dillenia philippinensis* Rolfe) cuttings on length of leaves 60 and 90 days after planting

Sources of katmon cuttings	60 DAP	90 DAP	SE	df	Lower CI	Upper CI
A1	2.72 ^a	32.8 ^{ab}	3.26	32	26.10	39.4
A2	3.06 ^a	34.5 ^a	3.26	32	27.85	41.1
A3	2.14 ^a	21.2 ^{bc}	3.26	32	14.60	27.9
A4	2.72 ^a	32.8 ^{ab}	3.26	32	26.10	39.4

A1-Plagiotropic Cloned; A2-Orthotropic Cloned; A3-Plagiotropic Seed Germinated; A4-Orthotropic Seed Germinated; Means that do not share the same letter are significantly different at 5% level of significant using Scheffe's Test (60 DAP). Estimated mean that do not share the same letter are significantly different at 5% level (90 DAP). Confidence level was set at 95% level. P-value adjustment was employed using Tukey's HSD method for comparing a family of 4 estimates.

elongation. The cloned cuttings are efficient and effective in propagating large-scale cultivation and conservation management for endangered species like katmon, increasing their population and maintaining their genetic traits (84). Several researchers have documented the use of cloning as an effective and rapid propagation for endangered and threatened species such as *Pterocarpus indicus*, *Elaeocarpus venustus* and *Allium microdictyon* (8587). In contrast, both orthotropic and plagiotropic seed-germinated cuttings showed delayed leaf production and elongation.

Hence, the study highlights efficient propagation and prioritizes cloning in the production of endangered species such as katmon over seed germinated as it produces more and longer leaves (88, 89). The minimal effect of root-inducing substances on leaf elongation reinforces the idea that such substances primarily influence root development. Consequently, successful propagation may depend more on controlled nursery conditions (light, humidity, temperature) than on the use of external stimulants (90).

Number of roots

No significant interaction was found between cutting sources and root-inducing substances in relation to root number after 90 days ($F(9, 47) = 0.733$, $p = 0.6759$, Table 11). However, the main effect of cutting source was significant ($F(3, 47) = 11.947$, $p < 0.001$). Pairwise comparisons revealed that plagiotropic cloned cuttings had the highest root count (EM = 37.2, SE = 3.3), followed by orthotropic cloned (EM = 30.6, SE = 3.3). These were significantly higher than both seed-germinated plagiotropic (EM = 17.7, SE = 3.3) and orthotropic (EM = 12.5, SE = 3.3)

cuttings (Table 12).

These findings indicate that the use of cloned katmon cuttings-either plagiotropic or orthotropic-results in significantly greater root development than seed-derived cuttings. No significant effects were found for root-inducing substances or their interaction with the cutting sources.

This supports the premise that selecting appropriate cutting sources is critical for optimal root development. Cloned cuttings demonstrated superior performance, potentially due to transcriptional regulation and hormonal balance (91), along with higher levels of endogenous auxins (92) and carbohydrate reserves (53, 93). These endogenous reserves provide the energy required for cell division, differentiation and root meristem development (94).

Length of roots

While no significant association was observed between different sources of katmon cuttings and the substances employed for root initiation, as indicated by $F = 0.663$, $p = 0.736 > 0.05$ (Table 11). The source of katmon cuttings did significantly impact the length of roots produced, $F(3,47) = 6.894$, $p < 0.0010$. Plagiotropic cloned (EM = 33.2, SE = 3.72) and orthotropic cloned (EM = 30.6, SE = 3.72) demonstrated notable differences when compared to orthotropic seed-germinated (EM = 11.5, SE = 3.72) as shown in Table 12.

The research indicates that both cloned katmon cuttings, whether plagiotropic or orthotropic, display significantly longer roots compared to seed-germinated katmon cuttings. Similar outcomes were observed across

Table 11. Two-way analysis of variance of number and length of roots of katmon (*Dillenia philippinensis* Rolfe) among different sources of katmon cuttings subjected to different levels of root initiating substances 90 days after planting

Sources of variation	df	Number of roots		Length of roots	
		F	p-value	F	p-value
SKC	3	11.947	<0.001*	6.894	0.001*
RIS	3	0.859	0.4722	0.653	0.587
SKC × RIS	9	0.733	0.6759	0.663	0.736
Error	32				
Total	47				

SKC – Sources of Katmon Cuttings; RIS- Root Initiating Substances; *Significant at 5% level using Aligned Rank Test

Table 12. Pairwise comparison of sources of katmon (*Dillenia philippinensis* Rolfe) cuttings on number and length of roots 90 days after planting

Sources of katmon cuttings	Number of Roots						Length of Roots					
	Estimated mean	SE	df	Lower CI	Upper CI	Grouping	Estimated mean	SE	df	Lower CI	Upper CI	Grouping
A1	37.2	3.3	32	30.53	44.0	a	33.2	3.72	32	25.68	40.8	a
A2	30.6	3.3	32	23.86	37.3	a	30.6	3.72	32	23.01	38.2	a
A3	17.7	3.3	32	10.95	24.4	b	22.7	3.72	32	15.09	30.2	ab
A4	12.5	3.3	32	5.78	19.2	b	11.5	3.72	32	3.93	19.1	b

A1-Plagiotropic Cloned; A2-Orthotropic Cloned; A3-Plagiotropic Seed Germinated; A4-Orthotropic Seed Germinated; Estimated mean that do not share the same letter are significantly different at 5% level. Confidence level was set at 95% level. P-value adjustment was employed using Tukey's HSD method for comparing a family of 4 estimates.

different root-inducing substances and the interaction effect between sources of cuttings and root-inducing substances did not yield any significant results regarding root length. Therefore, making informed choices when selecting cutting sources for longer roots provides crucial insights into plant survival and enhanced growth performance. Roots play a pivotal role in crop growth by absorbing water and nutrients from the soil. The results suggest that the functions of roots are crucial in different environments, with deeper roots, higher rooting depths and deep root ratios generally leads to greater absorption of water, while increased root length densities enhance nutrient uptake (95).

Both cloned plagiotropic and orthotropic exhibited consistent influence on the growth performance of the katmon, particularly in root elongation. Clone-propagated cuttings have inherent genetic uniformity and contain high endogenous auxin levels (Indole-3-acetic acid (IAA), enhancing root elongation and showing deeper and developed roots compared to seed germinated cuttings. IAA is involved in expanding the cell and root differentiation, an important trait for root primordia development (96, 97). Additionally, IAA is essential for woody species propagating through cuttings; it promotes the formation of adventitious roots (ARs) from cutting, thereby enabling successful propagation.

It enhances the survivability of the cuttings by forming ARs, especially in woody species propagating through cloning. It helps to control ARs for further growth and might produce auxins, making it an independent and self-sustaining growth process (98). Longer roots are very advantageous to the plant as they increase their nutrient and water absorption, which is vital for growth and development (95). Additionally, it can widely adapt in areas where water is scarce as deep-rooted plants increase their water uptake to sustain physiological and biochemical processes (99). The limited impact of RIS on the root indicates that sources of cuttings are superior in developing longer roots. Some studies documented that organic root-inducing substances are species-specific and may be less effective than other plant species. For instance, IAA a natural occurring plant hormone significantly influences the root count and length of *Eucalyptus grandis*. In contrast, *Eucalyptus globulus* did not exhibit significant influence (100).

Studies have shown that cloned cutting contains natural auxin (e.g., shoot apices), producing IAA, which is a primary endogenous auxin triggering root formation (56, 101). Auxin-producing tissues retain when the plant is cut, allowing polar auxin to be transported basipetally (toward the root) and creating localized auxin maxima that are vital for root formation and initiation. This activates the activated transcription factors (e.g., ARF), influencing root formation, initiation and elongation (57).

These results were supported by studies demonstrating similar observations on cloned cuttings yielding longer roots compared to seed-derived cuttings. A recent study reported that apple-cloned cuttings yielded longer roots than seed-derived cuttings primarily due to auxin retention and genetic uniformity identical to its parents (102). Meanwhile, *Eucalyptus* cuttings yielded extensive root systems as compared to seed-derived seedlings (103). The study highlights that cloned katmon cuttings are more efficient and effective in root

elongation over seed-germinated cuttings, thereby enhancing their chances of survivability in the field.

Conclusion

The study highlights that cloned katmon cuttings (both plagiotropic and orthotropic) consistently outperformed seed-germinated cutting across multiple parameters, significantly influencing leaf sprout number, leaf count, leaf length, root count and root length. Moreover, the study demonstrated that plagiotropic cloned katmon cuttings had the highest survivability of 90.0 % at 30 days, though survivability significantly declined across all treatments at 60 (46.67 %) and 90 (30.0 %) days. Cuttings treated with Aloe vera maintained the highest survivability (30.0%) even after 90 days.

Aloe vera also had the most favorable impact on sprout production at 30 days (mean=1.53), while cloned cuttings (plagiotropic-1.67 and orthotropic-1.48) produced significantly more leaf sprouts than seed-germinated counterparts, particularly at 60 days. Plagiotropic cloned cuttings remained impactful and exhibited superior root development in terms of the number (37.2) and length (33.2 cm) of the roots at 90 days.

Hence, plagiotropic cloned cuttings with Aloe vera treatment can be an effective and profitable strategy in improving plant propagation and increasing food production. Future studies are recommended to investigate the application of varying concentrations of Aloe vera to further optimize growth performance and potentially increase the genetic diversity of the katmon. This study opens the door for the development of sustainable propagation techniques that can enhance agricultural output in the long term. It also highlights the importance of the proper selection of cuttings and propagation techniques for katmon propagation to enhance diversity, population and conservation management.

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Authors' contributions

LT and GS crafted the proposals, coordination, critique, data gathering, analysed, interpret the data and manuscript writing. AB performed the study, monitored it and gathered the data. MR and KC participated in the study's design, critique and performed the statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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